VARIATION IN TEMPERATURE-SALINITY TOLERANCE BETWEEN TWO ESTUARINE POPULATIONS OF *PAGURUS LONGICARPUS* SAY (CRUSTACEA: ANOMURA)[1]

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Species living in an estuarine environment are normally subjected to frequent and variable fluxes in temperature and salinity. The hermit crab, *Pagurus longicarpus* Say, is found in the littoral area of estuaries from Nova Scotia to northern Florida, and from Sanibel Island, Florida, to Texas (Williams, 1965). The optimal salinity range for larval development through the megalopa stage is from 18.0 to 30.5‰ (Roberts, 1971). Optimal environmental conditions for growth, reproduction and survival of the postlarval stages, however, have not been clearly defined.

Tolerance defines the limits within which survival is possible (Alderdice, 1972). Fraenkel (1960) compared thermal tolerances of adult specimens of *P. longicarpus* collected from Woods Hole, Massachusetts, with two other intertidal zone inhabitants, *Limulus polyphemus* and *Littorina littorea*. Hermits could survive for one hour at 36° C, if allowed to recover for 24 hours at room temperature; 40° C was 100% lethal. More recently, Vernberg (1967) noted that *P. longicarpus* acclimated to 15° C survived longer than if acclimated to 5° C, when the environmental temperature was raised to 5, 10 or 15 degrees above the original acclimation temperature.

Kinne, Shirley and Meen (1963) found that the blood of *P. longicarpus* was isotonic with the environment at nine combinations of temperature and salinity. An increase in oxygen consumption as the salinity is lowered from approximately 18 of 5‰ (at 26 to 28° C) was reported for *P. longicarpus* by Nagabhushanam and Sarojini (1965). Vernberg and Vernberg (1972) investigated metabolic-temperature responses in latitudinally separated populations of the same species. Cold-acclimated crabs from Massachusetts and North Carolina populations consumed oxygen more rapidly than warm-acclimated animals. This apparent adaptational response was not observed in populations from Florida.

The euryhaline distribution of the adult and rather broad salinity tolerance of the larvae of *P. longicarpus* suggest that the species as a whole has the ability to adapt to most estuarine conditions. We investigated the tolerance of two populations from southern New Jersey to changes in salinity and temperature.

One population was collected from shallow flats at Grassy Sound Channel inside of Hereford Inlet on the Atlantic side of the Cape May peninsula (lat. 39°

01.72' north; long. 74° 48.10' west), where the average annual salinity is about 30‰. The other, from an intertidal area along Delaware Bay south of Pierces Point on the Cape (lat. 39° 04.5' north; long. 74° 54.9' west), lived in mean salinities of about 20‰. The two populations are not in direct contact, although gene flow between them might occur by larval transport around the tip of Cape May at the ocean entrance of the Bay, a distance of 15–20 miles, or by a slightly shorter route through the Cape May Canal to the north of Cape May Point.

Preliminary experiments indicated a differential survival when Hereford and Cape Shore populations were subjected to various combinations of temperature and salinity. Hence, acclimation work was done in the laboratory to investigate whether differential survival was influenced primarily by acclimation to different field salinities, or whether more complex intraspecific differences might be indicated.

METHODS

Hermits were collected four times during the fall of 1971, and twice in early 1972. They were gathered by hand, with no intentional bias for size, though turbid conditions at the Cape Shore often necessitated groping.

Cape Shore field salinities are consistently lower and more variable than those at Hereford Inlet. Records for several years at the Hereford station show that the average annual salinity is approximately 30‰, with a range from 28.1–32.6‰. The average annual salinity at the Cape Shore is approximately 20‰, range 15–27‰ (H. H. Haskin, Rutgers University, personal communication).

Field collections were maintained in shallow communal trays in the laboratory for at least 1–2 weeks before they were used in experiments. Discrete collections were kept segregated. Hermits were regularly fed *Crepidula fornicata, C. plana* and chopped *Mercenaria mercenaria*. Feeding was discontinued one week before temperature-salinity tolerance was investigated.

Wet room maintenance temperatures ranged from 16–20° C. Normal maintenance salinity for the Hereford population was 30 ± 1‰, and 22 ± 2‰ for the Cape Shore population. Water was changed as often as necessary, but at least once a week. If sufficient natural seawater was available, it was used for water changes, but appropriate dilutions of commercially-available sea salts (Instant Ocean, by Aquarium Systems, Inc.) were also used for these changes and for all experimental salinities. Salinity determinations were performed using a modification of Harvey's $AgNO_3$ titration method (Welsh and Smith, 1953).

Ten pairs of trials compared the tolerance of the two geographic populations. In each, six hermits were used per temperature-salinity combination. Hermits were isolated in 50 ml of artificial seawater in individual $51 \times 43 \times 39$ mm compartments in covered clear plastic boxes. The sex ratio in a group of six crabs depended on the availability of sexes in animals randomly removed from the shells prior to a trial. When possible, three of each sex were used. Crabs were induced to leave their gastropod shells by touching the tip of a soldering iron to the apex of the shell and allowing heat to drive the crab into a dish of cool water held directly beneath. Hermits were maintained in individual compartments for at least 3 days after removal, and those obviously injured were not used as experimental animals.

Hermits used in tolerance determinations were preserved after 96 hours and new crabs selected for the following trial. Length of the subcordate anterior shield was used as a size index (Markham, 1968).

Four temperatures and nine salinities were used to characterize temperature-salinity tolerance (Table I). Hermits from Hereford and Cape Shore fall collections maintained at their respective field salinities were used in Trials 1–4. In the first two trials, hermits were subjected to salinities from 5–25‰. In Trials 3 and 4, hermits were placed in salinities higher than 25‰. In Trials 5–7, we

TABLE I

Temperature-salinity combinations at which tolerance of Pagurus longicarpus *was investigated, and number of crabs used in each environmental combination (grand total = 1317).*

Temp °C	Salinity ‰								
	5.5	10.2	15.5	20.0	25.1	30.4	35.8	40.9	46.3
Cape Shore normal-acclimated to 22‰ (480 crabs: Trials 1, 2, 3, 4, 7)									
4	18	18	18	12	6	6	6	18	18
15	18	18	18	12	6	6	6	18	18
20	18	18	18	12	6	6	6	18	18
31	18	18	18	12	6	6	6	18	18
Hereford normal-acclimated to 30‰ (384 crabs: Trials 1, 2, 3, 4)									
4	12	12	12	12	12	6	6	12	12
15	12	12	12	12	12	6	6	12	12
20	12	12	12	12	12	6	6	12	12
31	12	12	12	12	12	6	6	12	12
Cape Shore acclimated to 30 ‰ (273 crabs: Trials 5, 6, 7)									
4	18	18	12	—	—	—	—	12	12
15	18	18	9	—	—	—	—	12	12
20	12	12	12	—	—	—	—	12	12
31	18	18	12	—	—	—	—	12	12
Hereford acclimated to 22‰ (180 crabs: Trials 5, 6)									
4	12	12	12	—	—	—	—	6	6
15	12	12	6	—	—	—	—	6	6
20	12	12	6	—	—	—	—	6	6
31	12	12	12	—	—	—	—	6	6

changed the natural acclimation salinity of 453 randomly-selected Hereford and Cape Shore crabs. Cape Shore crabs were held at 30‰, and Hereford at 22‰ for two weeks prior to determination of temperature-salinity tolerance. Trial 8 compared survival of 192 Cape Shore crabs acclimated to 30‰ for periods of one week and two months (not included in Table I).

Trials 9 and 10, using winter-collected animals, attempted a more precise resolution of salinity tolerance limits in crabs maintained at their respective field salinities. Hermits at 15° C were subjected to salinities of 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 35.0, 37.5, 40.0, 42.5 and 45.0‰.

At the start of each 4-day trial, crabs were abruptly subjected to the appropriate acute conditions. Survival was then checked at 1-hour intervals for the first 5 hours, again at 9 and 12 hours, and thereafter at 12-hour intervals until the end of the 4-day period. The relative activity and response of each survivor to stimuli was also noted at each time check, as an index of morbidity.

Onset of morbidity was characterized by a loss of normal coordination and equilibrium. Crabs under greater stress showed decreased locomotory activity. As death approached, regular gill bailer action ceased and the maxillipeds protruded. The abdominal area often became transparent, especially in low salinities.

Any animals showing relatively sustained motion of appendages such as gill bailer, pereiopods, pleopods or antennae, when lightly stimulated with a blunt probe, were regarded as being alive. Only when further irritability to moderate probing of the abdomen or ventral body surface ceased, was an animal considered dead. As a precautionary measure, the animal was not removed from its compartment until mortality was confirmed at the next time check.

Crabs surviving the 4-day period would generally survive for several additional days. Tolerance estimates in this study are based on cumulative per cent mortality by 96 hours, pooled for each set of hermits in Table I, and computer fitted to generate response surfaces (Figs. 1–4).

The surface fitted is best described by the quadratic form employed by Box and Youle (1955) and reviewed by Alderdice (1972): $Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_1X_2$, where Y is arcsin square root of cumulative per cent mortality, X_1 is temperature in °C, X_2 is salinity in ‰, B_0 a mean effect, B_1 a linear effect of temperature, B_2 a linear effect of salinity, B_{11} a quadratic effect of temperature, B_{22} a quadratic effect of salinity, and B_{12} an interaction effect of temperature and salinity. B-values were calculated from the experimental points and pooled observed mortality by the method of least squares. A separate equation was obtained for each acclimation condition.

Translation of the center of a response surface depicts a change in tolerance. Rotation of the principal axes of regression so that they are no longer parallel to those of temperature and salinity implies temperature-salinity interaction (Alderdice, 1972). Elongation of response isopleths (plasticity) occurs along the axis of the factor exhibiting the lesser effect.

Results

Mean size of P. longicarpus

Mean size of males from both Hereford Inlet and the Cape Shore was in all collections significantly larger ($\alpha = 0.01$) than the mean size of the respective females. Moreover, mean size of either sex of Hereford crabs was larger than mean size of animals of the same sex in each Cape Shore collection.

Estimation of optimal temperature-salinity combinations

The fitted response surfaces show regions of temperature and salinity where cumulative mortality at the end of 96 hours does not exceed 10% (Figs. 1–4). Normal-acclimated Cape Shore crabs show less than 10% cumulative mortality from 10–22° C, over a salinity range of 15–35‰ (Fig. 1). The response optimum
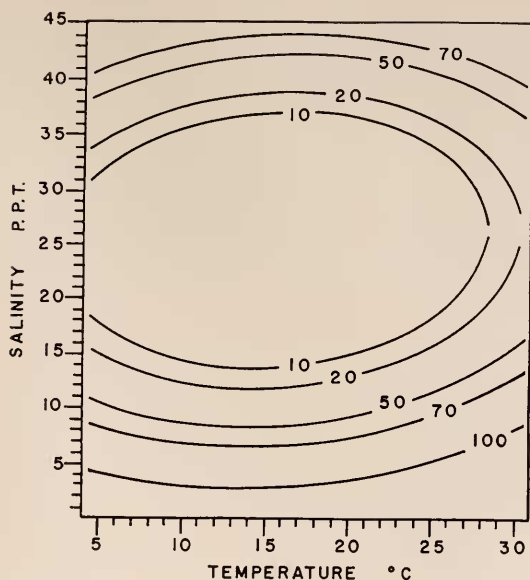
FIGURE 1.   Estimation of per cent mortality of Cape Shore hermits acclimated to a normal habitat salinity of 22‰ based on fitted response surface to observed mortality at 96 hours under thirty-six conditions of temperature and salinity ($Y = 2.31695 - 0.063953X_1 - 0.151087X_2 + 0.002455X_1^2 + 0.003134X_2^2 - 0.000483X_1X_2$).
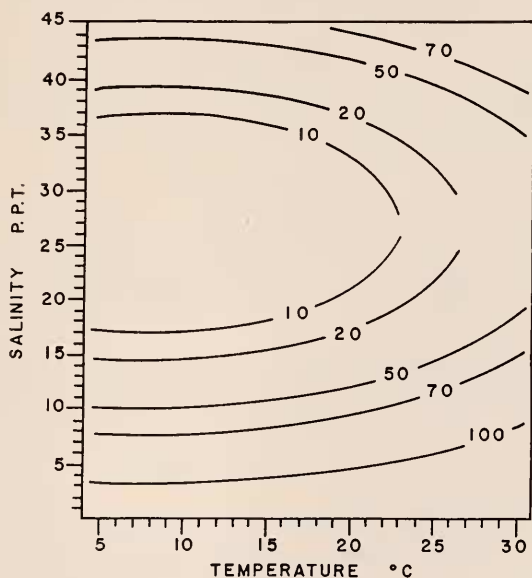

FIGURE 2.   Estimation of per cent mortality of Cape Shore hermits acclimated to a higher than normal salinity of 30‰ based on fitted response surface to observed mortality at 96 hours under twenty conditions of temperature and salinity ($Y = 1.96682 - 0.016500X_1 - 0.136434X_2 + 0.001089X_1^2 + 0.002557X_2^2 - 0.000038X_1X_2$).

should be located at the intersection of the geometric axes of the regression surface. Thus, optimum temperature for normal-acclimated Cape Shore crabs may be estimated to be 16° C, and optimum salinity at 25‰. Optimum conditions may be similarly found for each of the remaining three acclimation groups (Figs. 2–4).
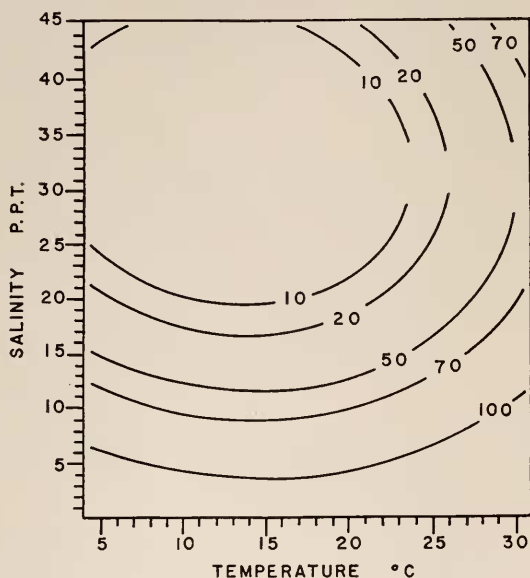

FIGURE 3. Estimation of per cent mortality of Hereford hermits acclimated to a normal habitat salinity of 30‰ based on fitted response surface to observed mortality at 96 hours under thirty-six conditions of temperature and salinity $(Y = 2.48732 - 0.079378X_1 - 0.12078X_2 + 0.002578X_1^2 + 0.001769X_2^2 + 0.000414X_1X_2)$.

Effect of acclimation on temperature-salinity tolerance

When Cape Shore crabs were acclimated for two weeks to a higher than normal salinity of 30‰, the primary effect was a decreased high temperature survival (Figs. 1 and 2). Conversely, Cape Shore crabs acclimated to this high salinity realized better low temperature survival than crabs maintained in a normal habitat salinity. The response surface isopleths for Cape Shore animals acclimated to a higher than normal habitat salinity translated left almost 6° C along the temperature axis (Figs. 1 and 2). Despite euryplastic temperature tolerance, populations from the Cape Shore retained a salinity optimum relatively unresponsive to changes in acclimation salinity (Figs. 1 and 2).

Similar but less marked translations in temperature tolerance were observed in Hereford crabs acclimated to high and low salinity. In Hereford populations, however, the position of optimum salinity was obviously influenced by acclimation salinity (Figs. 3 and 4).

If temperature tolerance of Cape Shore crabs is now compared with Hereford crabs (Figs. 1 and 3), Cape Shore crabs showed wider temperature tolerance

than their Hereford counterparts. When acclimated to the same salinity, varia-
tion in temperature tolerance between Cape Shore and Hereford crabs decreased,
but Cape Shore crabs still survived wider extremes of temperature than did iden-
tically acclimated Hereford crabs. Differential temperature tolerance is more pro-
nounced at 22‰ acclimation (Figs. 1 and 4) than at 30‰ (Figs. 2 and 3).

In interpreting temperature tolerance, one must keep in mind that a popula-
tion acclimated to higher salinity will survive better at high salinity extremes,
regardless of the temperature. Conversely, at any combination of temperature
and low salinity, a low salinity acclimated group should have a survival advantage.
Interaction of temperature and salinity is minimal and independent of acclimation
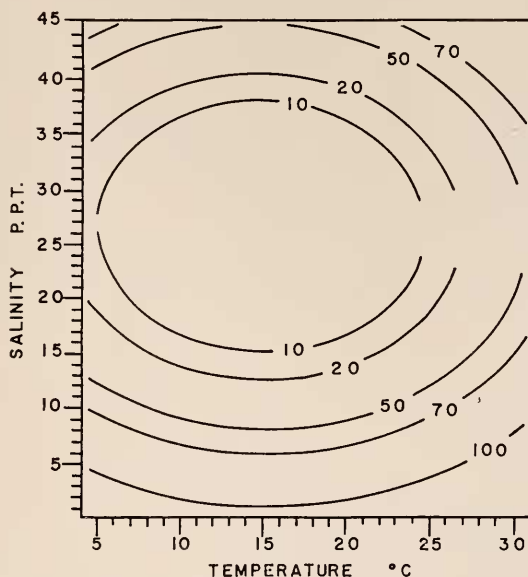salinity.

FIGURE 4. Estimation of per cent mortality of Hereford hermits acclimated to a lower
than normal salinity of 22‰ based on fitted response surface to observed mortality at 96 hours
under twenty conditions of temperature and salinity $(Y = 2.302 - 0.089643X_1 - 0.120942X_2 + 0.002916X_1^2 + 0.02255X_2^2 + 0.000112X_1X_2)$.

Finally, independent of changes in acclimation salinity, low temperatures
near 4° C represented more favorable conditions for *P. longicarpus* than high
temperatures near 31° C.

Additional factors and their effects

Seasonal variation in time to reach LD-50 was negligible in both optimum and
extreme temperature-salinity combinations, and seldom varied by more than 12
hours when populations collected at different times during 1971–72 were subjected
to any of the temperature-salinity combinations.

Cape Shore crabs with only one week acclimation to higher than normal
salinity showed tolerance intermediate between that of a normal-acclimated popula-
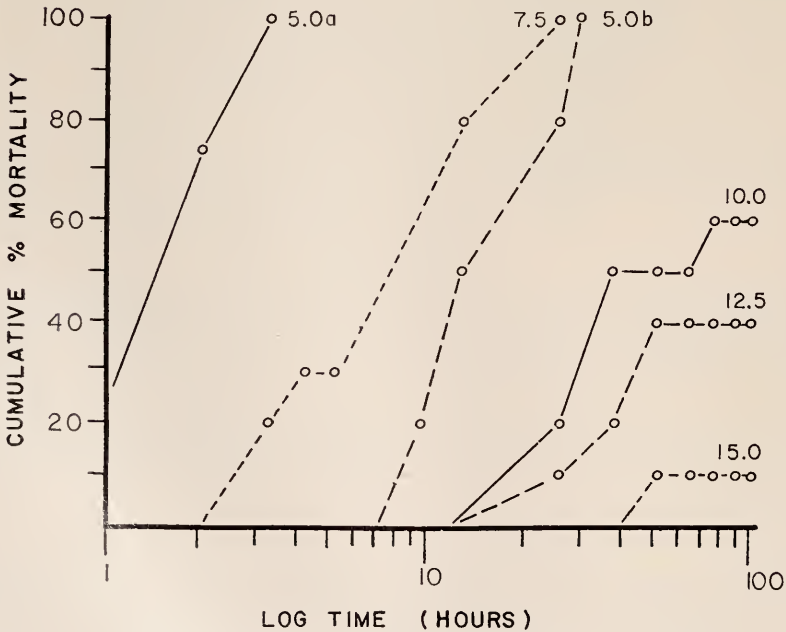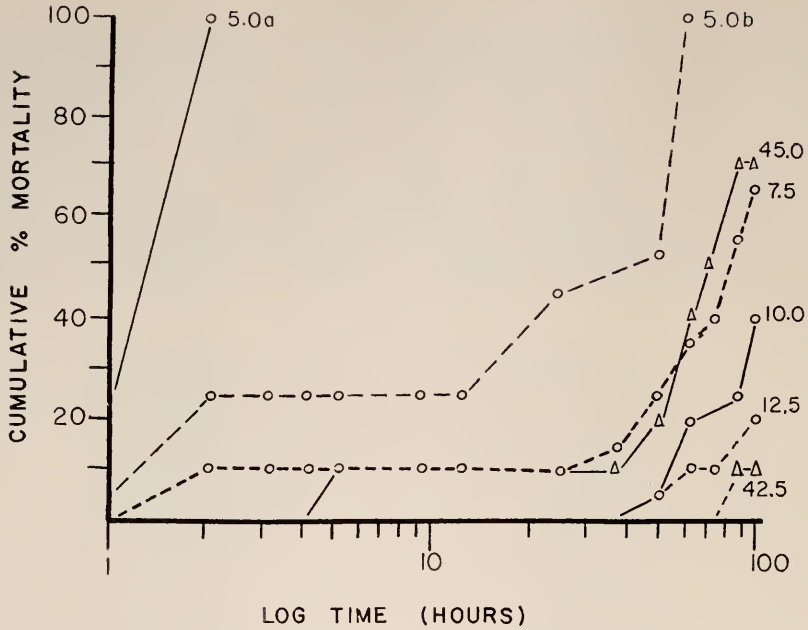
FIGURE 5. Cumulative per cent mortality of Cape Shore hermits acclimated to a normal habitat salinity of 22‰ by 96 hours at 15° C. Only those salinities in which mortality occurred are plotted (see text) (5.0a = acute transfer to 5.0‰; 5.0b = 96-hour acclimation to 15‰ before transfer to 5.0‰).

tion and groups held for two weeks under higher than normal salinity. There were no consistent differences in cumulative mortality after 96 hours between two-week and two-month acclimated groups.

Neither sex nor size appeared to influence mortality in *P. longicarpus,* although both in general often influence tolerance (Alderdice, 1972). In all trials, roughly equal percentages of each sex remained at LD-50, and when the mean size of living *versus* dead crabs at 96 hours was computed, ratios approached unity in groups of more than ten crabs.

Although no obvious selection for size or sex was noted, mortality seldom occurred at random throughout a 96-hour period. Rather, in both Hereford and Cape Shore populations, a large increase in cumulative mortality often occurred within a short time, especially at extremes of salinity (Figs. 5 and 6).

At less harsh salinity conditions, animals often showed some initial mortality, followed by increased tolerance, which caused leveling off of cumulative mortality (Fig. 5) as a degree of resistance adaptation was acquired (Kinne, 1970). Often, a second critical period would then be reached during which cumulative mortality increased rapidly until LD-100 was reached.

When salinity transitions are less abrupt than those employed in the present study, the nature of the response surfaces may be altered. For example, broad but gradual changes in salinity may postpone time to LD-50. A postponement of LD-50 was noted when animals were acclimated for four days to 15‰ before being transferred to 5‰ (Figs. 5 and 6). Note that LD-100 was nevertheless reached in both cases.

Cumulative mortality at 48 and 96 hours

Tolerance estimates for adult specimens of *P. longicarpus* have been somewhat arbitrarily based on cumulative mortality at 96 hours. A somewhat shorter time period might have been chosen, since most of the mortality usually occurred by 48 hours. For hermits from either population, areas of temperature and salinity enclosed by a 20% cumulative mortality isopleth were only slightly more compressed at the end of 96 hours. In experiments with a shrimp, *Penaeus aztecus,* Zein-Eldin and Aldrich (1965) demonstrated that it showed a degree of tolerance only slightly less over periods of 28 days when compared to 24 hours.

Discussion

Roberts' (1971) estimates of salinity tolerance in the larval stages of *P. longicarpus* cover a period from hatching through the megalopa stage. When supplemented by estimates of adult tolerance proposed in the present study, they allow prediction of an optimum salinity range for most life-history stages. Ovigerous females used in Roberts' study were collected from a beach at Gloucester Point, Virginia. Larvae were hatched and maintained at 20° C in water of 20‰, and development at the same temperature followed at 10, 15, 20, 25 and 30‰.

FIGURE 6. Cumulative per cent mortality of Hereford hermits acclimated to a normal habitat salinity of 30‰ by 96 hours at 15° C. Only those salinities in which mortality occurred are plotted (see text) (5.0a = acute transfer to 5.0‰; 5.0b = 96-hour acclimation to 15‰ before transfer to 5.0‰).

Roberts concluded that tolerance of reduced salinity is the same for all four zoeal stages, and slightly less for the megalopae. Over a salinity range from 18.0–30.5‰, there were no significant differences in either zoeal mortality, hatching time, or intermolt duration. In comparison, over a 15–36‰ salinity range, normal-acclimated Cape Shore adults are estimated to show less than 10% mortality in a 96-hour period (Fig. 1). Hereford adults acclimated to the same low salinity show 10% mortality over a nearly equivalent 17–36‰ range (Fig. 4). If Virginia adults behave similarly, optimum salinity range at 20° C for adult specimens of *P. longicarpus* is apparently somewhat wider than optimum larval range. Larval development should be followed at salinities above 30‰, however, as this is an unlikely upper limit.

In the preceding discussion, one candidly assumed that tolerance of Virginia larvae could be compared with New Jersey adults acclimated to the same salinity. However, in the present study, adults from two New Jersey populations were acclimated for two weeks to the same salinity, and tolerances were not identical.

If the physiological basis for differential survival between adult Hereford and Cape Shore populations resided solely in the different natural-acclimation salinities of 22‰ and 30‰, equivalent acclimations would be expected to produce identical temperature-salinity tolerance. However, since acclimation of both populations to the same salinity consistently produced tolerance intermediate to that of natural-acclimated populations, more complex interactions or intraspecific physiological differences may determine tolerance.

The Cape Shore hermits inhabit a more extensive network of shallow flats and bars than is found at Hereford Inlet. Both temperature and salinity are more variable here. Proximity to fresh water sources influences salinity variation, while the shallow nature of the area can permit summer temperatures to rise above those of the more ocean-buffered system at Hereford Inlet. Water temperature may vary as much as 5° C during a summer tidal cycle (Hendler and Franz, 1971). In winter, Cape Shore crabs may experience lower temperatures since at least some are known to remain in the intertidal zone under winter conditions. In contrast, the Hereford population migrated completely out of the intertidal area, so that by January, none remained on exposed flats.

Because Cape Shore crabs are presumably subjected to seasonally wider extremes of temperature, a degree of selection for temperature resistant forms may occur in this population. Unless high temperature tolerance is normally present in a population, however, laboratory acclimation to a low salinity apparently will not increase temperature tolerance. In populations of Hereford crabs acclimated to 22‰, temperature tolerance was not markedly elevated.

Salinity-dependent temperature tolerance exhibited by Cape Shore crabs may derive from osmotic considerations. Crabs naturally-acclimated to low salinities, when subjected to higher than normal salinity acclimation stress, may be spending energy in hyporegulating, which makes them more susceptible to temperature stress. In support of this suggestion, preliminary unpublished investigations by one of us (D. C. B.) do indicate a narrow range of active osmoregulation in *P. longicarpus*. Blood of adults acclimated to 30‰ was demonstrably hypotonic in environmental salinities between 30 and 40‰.

Crabs acclimated to high salinity may find low salinity environments equally deleterious. Larvae reared at 20‰ and then placed into 10‰ showed significantly greater mortality than those placed into 30‰ (Roberts, 1971). Adults responded similarly. *P. longicarpus* increased its oxygen consumption in low salinities at a temperature of about 27° C (Nagabhushanam and Sarojini, 1965). This was interpreted as an increase in osmoregulatory work, although alternative explanations are possible, *e.g.*, increased locomotor activity (Lockwood, 1967, page 150), or scaphognathite activity. A European hermit crab, *Eupagurus bernhardus*, showed 15.4% swelling when subjected for one hour to a hypotonic environment. Swelling was subsequently relieved by increased rates of urine production (Davenport, 1972).

The response surfaces of *P. longicarpus* can be compared with those fitted for another decapod. Haefner (1969) used twelve different combinations of temperature and salinity to generate a response surface for the sand shrimp, *Crangon septemspinosa*. This species inhabits estuarine environments similar to those of *P. longicarpus*, and in fact, may be collected in the intertidal zone at the Cape Shore and Hereford Inlet. Price (1962) collected this shrimp throughout Delaware Bay in salinities which ranged from 4.4 to 31.4‰. Response surface isopleths show quite similar elongation and distribution, and salinity exerts a greater effect on tolerance than does temperature. As with *P. longicarpus*, lower or higher temperatures reduce the salinity range in which maximum survival can be expected.

Although a multifactor analysis was designed to study tolerance of *Crangon*, shrimp were collected from only a single geographical area out of the total species range, and no changes in acclimation salinity were attempted. As the present study has illustrated, estimates of tolerance made independently of acclimation work may be of limited value, and response surface models generated from such preliminary data should probably be applied only to populations naturally-acclimated to similar salinities.

SUMMARY

1. A hermit crab, *Pagurus longicarpus* Say, was collected from two sites in southern New Jersey at approximately the same latitude but on different sides of the Cape May peninsula. Those from Hereford Inlet lived in mean salinity of 30‰, and Delaware Bay Cape Shore hermits lived in 20‰.

2. Adults from both populations were acclimated for two weeks to 22‰ and 30‰ and were then subjected to 20–36 combinations of temperature and salinity. Cumulative mortality after 96 hours was used to fit response surfaces for estimating tolerance.

3. Hereford and Cape Shore populations differed in temperature-salinity tolerance. Cape Shore crabs showed wider temperature tolerance than identically-

acclimated Hereford crabs, while the latter survived greater variation in salinity. Apparently, different acclimation salinities are not alone sufficient to explain differential tolerance.

4. Salinity tolerance of adults from New Jersey is compared with tolerance of zoeae and megalopae from previous studies in Virginia. At 20° C, zoeal salinity tolerance is identical from 18.0–30.5‰. At the same temperature, estimated optimal salinity range for adults is 15–36‰.

LITERATURE CITED

ALDERICE, D. F., 1972. Factor combinations. Pages 1659–1772 in O. Kinne, Ed., *Marine Ecology, Vol. 1, Pt. 3.* Wiley-Interscience, New York.

BOX, G. P., AND P. V. YOULE, 1955. The exploration and exploitation of response surfaces: An example of the link between the fitted surface and the basic mechanism of the system. *Biometrics,* **11** : 287–323.

DAVENPORT, J., 1972. Volume changes shown by some littoral anomuran Crustacea. *J. Mar. Biol. Ass. U. K.,* **52** : 863–877.

FRAENKEL, G., 1960. Lethal high temperatures for three marine invertebrates: *Limulus poly-phemus, Littorina littorea,* and *Pagurus longicarpus. Oikos,* **11** : 171–182.

HAEFNER, P. A., JR., 1969. Temperature and salinity tolerance of the sand shrimp, *Crangon septemspinosa* Say. *Physiol. Zool.,* **42** : 388–397.

HENDLER, G., AND D. R. FRANZ, 1971. Population dynamics and life history of *Crepidula convexa* Say (Gastropoda: Prosobranchia) in Delaware Bay. *Biol. Bull.,* **141** : 514–526.

KINNE, O., 1970. Temperature. Pages 321–514 in O. Kinne, Ed., *Marine Ecology, Vol. I, Pt. 1.* Wiley-Interscience. New York.

KINNE, O., E. K. SHIRLEY, AND H. E. MEEN, 1963. Osmotic responses of hermit crabs (*Pagurus longicarpus* Say) exposed to various constant temperatures and salinities. *Crustaceana,* **5** : 317.

LOCKWOOD, A. P. M., 1967. *Aspects of the Physiology of Crustacea.* W. H. Freeman and Co., San Francisco, 328 pp.

MARKHAM, J. C., 1968. Notes on growth-patterns and shell-utilization of the hermit crab *Pagurus bernhardus* (L.). *Ophelia,* **5** : 189–205.

NAGABHUSHANAM, R., AND R. SAROJINI, 1965. The influence of low salinity upon the oxygen consumption in the hermit crab, *Pagurus longicarpus* Say. *Sci. Cult.,* **31** : 43–44.

PRICE, K. S., JR., 1962. Biology of the sand shrimp, *Crangon septemspinosa,* in the shore zone of the Delaware Bay region. *Chesapeake Sci.,* **3** : 244-255.

ROBERTS, M. H., JR., 1971. Larval development of *Pagurus longicarpus* Say reared in the laboratory. II. Effects of reduced salinity on larval development. *Biol. Bull.,* **140** : 104–116.

VERNBERG, F. J., 1967. Some future problems in the physiological ecology of estuarine animals. Pages 554–557 in G. H. Lauff, Ed., *Estuaries.* Publication No. 83, American Association for the Advancement of Science, Washington, D. C.

VERNBERG, W. B., AND F. J. VERNBERG, 1972. *Environmental Physiology of Marine Animals.* Springer-Verlag, New York, 346 pp.

WELSH, J. H., AND R. I. SMITH, 1953. *Laboratory Exercises in Invertebrate Physiology.* Burgess, Minneapolis, 126 pp.

WILLIAMS, A. B., 1965. Marine decapod crustaceans of the Carolinas. *U. S. Fish. Wild. Serv., Fish. Bull.,* **65** : 1–298.

ZEIN-ELDIN, Z. P., AND D. V. ALDRICH, 1965. Growth and survival of postlarval *Penaeus aztecus* under controlled conditions of temperature and salinity. *Biol. Bull.,* **129** : 199-216.